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TITLE: T cells and Rejection in Vascularized Composite Allotransplants

PRINCIPAL INVESTIGATOR: Bohdan Pomahac, MD

CONTRACTING ORGANIZATION: Brigham and Women's Hospital, Boston, MA

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Immune rejection is a major barrier to wider implementation of vascular composite allografts (VCAs) that hold great promise for restoring function in American service members, who have suffered devastating traumatic injuries. Despite systemic immunosuppression, T cell mediated rejection (TCMR) occurs much more frequently in VCA than in solid organ transplants, likely due to the significant number of donor T cells that survive in the allografts. This study will use banked tissues from VCA patients to comprehensively analyze the contributions of donor versus recipient T cells in VCA rejection. Another question that will be addressed is whether sentinel flaps, transplanted concomitantly with the allograft from the same donor to a distant anatomical site, or circulating levels of clonally expanded T cells, are useful as reliable markers for VCA rejection. IRB and HRPO approval for this project has been obtained.

15. SUBJECT TERMS
None listed.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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1. INTRODUCTION:

Immune rejection is a major barrier to wider implementation of vascular composite allografts (VCAs) that hold great promise for restoring function in American service members, who have suffered devastating traumatic injuries. Despite systemic immunosuppression, T cell mediated rejection (TCMR) occurs much more frequently in VCA than in solid organ transplants, likely due to the significant number of donor T cells that survive in the allografts. This study will used banked tissues from VCA patients to comprehensively analyze the contributions of donor versus recipient T cells in VCA rejection. Another question that will be addressed is whether sentinel flaps, transplanted concomitantly with the allograft from the same donor to a distant anatomical site, or circulating levels of clonally expanded T cells, are useful as reliable markers for VCA rejection.

2. KEYWORDS:

Vascular composite allograft, rejection, biomarker, T cell

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major Task 1: Regulatory approval by sponsor and institution. IRB approval estimated at Month 1; Actual approval achieved at Month 3. HRPO approval estimated to occur in Month 3 was completed in late 2019. Current percentage of completion is 100%.

Major Task 2: Activities for Specific Aim 1.
Subtask 1: High throughput TCR sequencing (HTS) of donor and recipient tissues from 6 face transplant patients during rejection and non-rejection. During month 12, samples from 3 patients were prepared to be sent for high throughput sequencing; current percentage of completion is 75%. This work will continue in the laboratory of Dr. Rachel Clark.
Subtask 2: Determine functional phenotypes of clonally expanded donor and recipient T cells using single nucleus RNA sequencing (sNucSeq), using samples from 7 face transplant patients. We have optimized this novel protocol in skin, percentage of completion is 30%. We have data provided for our first optimization of sNucSeq in skin – a novel approach that has never been performed in cryopreserved skin. This work will continue in the laboratory of Dr. Rachel Clark.
Subtask 3: Validation of findings (HTS and sNucSeq) from face transplant cohort in additional VCA type (using tissue samples from 3 upper extremity transplant patients). Current percentage of completion is 50%. This work will continue in the laboratory of Dr. Rachel Clark.

What was accomplished under these goals?

Under Major Task 1, we obtained IRB and HRPO approval in late 2019. Current percentage of completion: 100%

Under Major Task 2, samples from 3 patients were prepared to be sent for high-throughput TCR sequencing. Personnel completed training in collaborator lab to begin optimizing sample digestion for sNucSeq. Collaboration delayed due to Coronavirus pandemic. We have optimized the novel preparation/digestion of skin to exract nuclei from frozen tissues in a manner suitable for single nucleus RNA sequencing. We will continue to optimize this protocol and perform the assay on samples from vascular composite allograft patients. Single Cell RNA sequencing was performed using Demuxlet, which identified donor vs. recipient cells by SNP genotyping. These samples are undergoing analysis by our collaborating bioinformaticians. This work will continue in the laboratory of Dr. Rachel Clark.

What opportunities for training and professional development has the project provided?

This project has provided training for the postdoctoral fellow in Dr. Clark's laboratory, William J. Crisler, to learn new lab skills. Specifically, he has been trained on analyzing high throughput sequencing data using the software of Adaptive Biotechnologies as well as the NanoString nSolver software. He also has been trained to optimize single nucleus sequencing in skin, a novel human tissue for this protocol.

How were the results disseminated to communities of interest?

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?

Nothing to Report

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Since we have now performed our optimization of the sNucSeq protocol, we can now perform this on our face transplant samples. Current optimization efforts are underway to extend our sNucSeq to include valuable sequencing data of the T cell repertoire in samples. These optimization efforts are ongoing and will continue in Dr. Rachel Clarks laboratory.

We have successfully performed the first nuclear digest from frozen, OCT-banked skin. We have performed three separate successful sequencing optimization experiments, yielding promising results. The skin samples used for optimization were from CTCL, a type of skin cancer. Moving forward, Dr. Clarks group will build upon these valuable, paradigm-shifting technological advances to continue in trying to capture the TCR sequencing from single nuclear sequencing in OCT-banked skin using homemade primers. We will optimize the homemade primers to attempt amplification of pre-mRNAs to capture TCR library from nuclear digest of human skin.

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

IRB and HRPO approvals took longer than expected. Shortly after receiving the HRPO approval, Brigham & Women's shut down in-person research activity as a result of the coronavirus pandemic. Personnel continued working on this project remotely and for the past several months have been back on-site in the laboratory and staff are addressing Major Tasks 2 and 3 and making significant progress. Dr. Clark's team will still be working on all aspects of this project in her laboratory and our closure of this award will have no bearing on her ability to proceed with this important study.

Changes that had a significant impact on expenditures

Less funds have been spent due to approval delays and the coronavirus pandemic which slowed down our timeline for this project, but experiments to address Major Tasks 2 and 3 have since resumed and are making good progress. Dr. Pomahac has left the institution (Brigham and Women's Hospital) as of July 31, 2021 for a new position as Chief of Plastic Surgery at Yale. Since his departure is only a few months prior to the project end date, the decision was made to close out his portion of this project and return the unspent funds. The project will continue in the laboratory of Dr. Rachel Clark to bring all of the assigned tasks to completion.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

IRB (Protocol # 2016P002185): Closed as of 08/03/2021

Significant changes in use or care of vertebrate animals

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS:

- **Publications, conference papers, and presentations**

  Journal publications.

  Nothing to Report

  Books or other non-periodical, one-time publications.

  Nothing to Report

  Other publications, conference papers and presentations.

  Nothing to Report

- **Website(s) or other Internet site(s)**

  Nothing to Report

- **Technologies or techniques**

  Nothing to Report

- **Inventions, patent applications, and/or licenses**

  Nothing to Report

- **Other Products**

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Rachael Clark, MD PhD
Project Role: Principal Investigator
Nearest person month worked: 1.2
Contribution to Project: Dr. Clark provided scientific oversight and provided feedback and support on regulatory and protocol submissions.

Name: William J. Crisler, PhD
Project Role: Research Fellow
Nearest person month worked: 10.8
Contribution to Project: Dr. Crisler has worked on regulatory submissions as well as preparing the samples and procedures for Major Task 2. He has also performed the analysis for Major Task 2 subtask 1.

Name: Bohdan Pomahac, MD
Project Role: Principal Investigator
Nearest person month worked: 1.2
Contribution to Project: Dr. Pomahac provided scientific oversight and provided feedback and support on regulatory and protocol submissions.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Rachael Clark, MD, PhD
None

Bohdan Pomahac, MD

Recently closed support: Novel strategies to improve immunomodulation and noninvasive clinical monitoring in VCA – project ended 7/31/2021

What other organizations were involved as partners?

Organization Name: Broad Institute
Location of Organization: Cambridge, MA
Partner's contribution to the project: Partner's staff has facilitated our performance of sequencing (sNucSeq) on human skin samples so that we have begun to perform sNucSeq on VCA samples.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES: